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Symptomatology of purple blotch disease of onion and exploration of fungicides, phytoextract and bio-agents against causal fungus *Alternaria porri*

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ABSTRACT

In field, Onion blight / purple botch symptoms development starts from tip of the older leaves as small, whitish, sunken, oval shaped lesions which later On became elliptical or oblong, which brown to purple at the centre and surrounded by a light brown area. Further, the lesions coalesce and spread rapidly on leaf blade and affected leaves show drying from tip downward. The leaves break at the affected area and hang down. Concentric light and dark rings were observed on the infected leaves. In older spots, the conidia were produced abundantly on bunch of conidiophores. Brown lesions with reddish-purple margins resembling bull's eye were also noticed. Affected leaves and stems turn yellowish white and die. On inoculated plant, blight symptoms were visible within 4 - 5 days of inoculation as small, water soaked sunken more or less oval shaped spots on leaves whichenlarged, became elliptical to oblong, zonate and turned brown to purple surrounded by yellow halo extending upwards and downwards with light to dark concentric rings. Lesions usually girdle leaves, causing them to fall over or hang down. Within 25 - 27 days after inoculation the affected plant showed complete drying of leaves. Under natural / field and artificial inoculation, symptoms induced by *A. porri,* causing blight (purple blotch) were recorded. All the fungicides tested *in vitro* against the pathogen were found effective in inhibiting the mycelial growth. Propiconazole 25% EC (0.1%) and combi product of iprodione 25% + Carbendazim 25% WP (0.1 and 0.05%) completely inhibited the mycelial growth of *A. porri*. Among the different plant extract testet against *A. porri* maximum per cent inhibition of mycelial growth was achieved with 10 per cent Cinnamon extract (72.22%), followed by Soapnut (64.77%) and Jatropha (64.44%). Among the various bio-agents tested, complete inhibition of *A. porri* was achieved by *Trichoderma hargianum* and *Aspergillus niger,* followed by *T. viride* and *pseudomonas flourescens.*

Key words: *Alternaria porri,* symptoms, fungicides, phytoextract, bio-agent, inhibition.

Abbreviations: Ap - *Alternaria porri,* An - *Aspergillus niger,* NSKE - Neem seed karnel extract, Sc - *Saccharomyces cerevisiae,* Th - *Trichoderma harzianum,* Tv - *Trichoderma viride.*

1. INTRODUCTION

Onion (*Allium cepa* L.) a bulbous, biennial herb, rightly called as "Queen of kitchen", is one of the most important vegetable crops grown throughout the world. Among the various foliar diseases of onion, blight (purple blotch) caused by *Alternaria porri* is one of the most destructive diseases, commonly prevailing in almost all onion growing pockets of the world, causing heavy loss in onions, under field conditions. The pathogen *Alternaria porri* destructs the leaf tissues which hinder the stimulus for bulb initiation and delays bulbing and maturation. Severe attack on flowering onion can completely girdle flower stalks with necrotic tissue, causing their collapse and total loss of seed production capacity. In Konkan region of Maharashtra, white onion is cultivated on commercial scale in some pockets of Raigad district and the crop severely affected by blight (purple blotch) disease. The disease now a days becoming major hurdle in successful cultivation of onion in Konkan region. Keeping in view, economic importance and yield losses caused by blight (purple blotch), the present investigations were under taken.

2. MATERIALS AND METHODS

2.1. Symptomatology

2.1.1. Under natural / field conditions

For studying blight (purple blotch) of symptoms, the field grown onion crop was frequently inspected and observed the disease symptoms expressed. The symptoms produced were critically compared with the symptoms produced on the artificially inoculated host plants under pathogenicity test. The symptoms were recorded till complete blightening of the leaves.

2.1.2. Under artificial / inoculated conditions

Twenty five days old, healthy and vigorously growing onion seedlings of 'Alibag local' variety were selected and planted in plastic pots filled in with sterilized potting mixture. Twenty days after transplanting in pots, the seedlings were sprayed with *A. porri* fungal inoculum by pin prick method and then such inoculated plants were placed in polythene moisture chamber to provide requisite amount of relative humidity. Inoculated plants were watered regularly. The symptoms were recorded from initiation to blightening of leaves.

2.2. *In vitro* evaluation of the fungicides against *Alternaria porri*

Nine different fungicides were tested against the test pathogen, applying Poisoned Food Technique (Nene and Thapliyal, 1993). Three replications per treatment were maintained. The observations for colony diameter and sporulation were recorded until whole of the plate in control treatment was fully covered with mycelial growth. Per cent inhibition of growth of the test fungus was calculated (Horsfall, 1956).

2.3. Evaluation of phytoextracts against *A. porri*

The aqueous plant extracts of neem (*Azadirachta indica*), nilgiri (*Eucalyptus globulus*), cinnamon (*Cinnamomum verum*), caster (*Ricinus communis*), clove (*Syzygium aromatium*), cassia (*Cinnamomum aromaticum*), jatropha (*Jatropha curcas*), sarpgandha (*Rauvolfia Serpentina*), soapnut (*Sapendus tripholiarus*) and garlic (*Allium sativum*) were as per the method described by Bhatti (1988). The effect of plant extracts on mycelial growth was studied by 'Poisoned Food Technique' (Nene and Thapliyal, 1993). All the plant extracts were tested at 10 per cent concentration against the test pathogen using oat meal agar as a basal medium. The experiment was planned with CRD and all the treatments replicated trice. The observations on colony diameter of the fungus were recorded when untreated control Petri plate was fully covered with mycelial growth of the test fungus.

2.4. Evaluation of bio-agents against *A. porri*

An *in vitro* study was conducted by dual culture method with five bioagents *viz*., *Trichoderma harzianum, T. viride* and *Aspergillus niger,* two strains of yeast *(Saccharomyces cerevisiae)* and *Pseudomonas fluorescens* (bacterial bio-agent). The trial was conducted in two possible ways. In the first case, the test fungus was placed at the centre of Petri plates surrounded by test bioagents and in second, the test fungus was placed at the periphery of Petri plates and bioagent at the centre. The bioagents were separately grown on PDA and NA in Petri plates. The fungal discs of 5 mm diameter were placed in such a way that both the fungi would get equal opportunity for their growth. For testing of *P. fluorescens* and yeast cultures, Bangal method and streaking were followed. Each treatment was replicated three

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times. The plates were then incubated at room temperature (27 ± 2°C) for seven days. The observations on colony diameter of the test fungus were recorded seven days after inoculation, when the control Petri plate was fully covered with mycelial growth of the test pathogen. Per cent mycelial growth inhibition was calculated (Horsfall, 1956).

3. RESULTS

3.1. Symptomatology

3.1.1. Natural / field conditions

The initial symptoms development starts from tip of the leaves particularly on older leaves and then extends to young ones. On infected leaves small, whitish, sunken, oval shaped lesions were found which later became elliptical or oblong. The lesions were brown to purple at the centre surrounded by a light brown area. Change in colour of leaves was started from centre of the lesion forming a yellow halo around lesions, which extended above and below the lesions. Further, the lesions coalesce and spread rapidly on leaf blade and affected leaves showed drying from tip downward. Many a times such as leaves break at the affected area and hang down. Concentric light and dark rings were observed on the infected leaves, when the affected lesion enlarges. In severe cases, blotches were enlarged up to 3 - 4 inches long and covered with conidia. In older spots, the conidia were produced abundantly on conidiophores. Brown lesions with reddish-purple margins resembling bull's-eye were also noticed. Such purplish bull's eye shaped lesions were also observed on whitish scales at base of onion plant. Affected area later became blackish to purplish due to production of conidia, leaves and stems turn yellowish white and die.

3.1.2. Artificial / inoculated conditions

On artificially inoculated onion plant, blight symptoms were visible within 4 - 5 days of inoculation as small, water soaked, sunken and more or less oval shaped spots on leaves. As the disease advanced, the lesions enlarged, became elliptical to oblong, zonated and turned brown to purple surrounded by yellow halo extending upwards and downwards. On the infected area, light to dark concentric rings were developed. The inoculated plants showed yellowing of leaves on 14th day of inoculation. Lesions usually girdle leaves, causing them to fall over or hang down. Within 25 - 27 days after inoculation the affected plant showed complete drying of leaves.

3.2. *In vitro* efficacy of the fungicides

All of the ten fungicides screened *in vitro* for their efficacy were found effective against *A. porri*. However, the results presented in Table 1 revealed that propiconazole 25% EC (0.1 %) and the combi fungicide iprodione 25% + carbendazim 25% WP (0.1 and 0.05 %) completely inhibited the mycelial growth of *A. porri.* Difenconazole 25% EC (0.1 %), Tebuconazole 25.9% EC (0.1 %) and combi fungicide carbendazim 12% + mancozeb 63% WP (0.2 %), showed 88.00, 85.77 and 84.66 per cent inhibition, respectively. Mancozeb 75% WP (0.25 %) and copper oxychloride 50% WP (0.2 %) resulted in 79.77 and 76.33 per cent inhibition over control, respectively with no conidia formation. Comparatively minimum inhibition was recorded with 0.1 per cent carbendazim 50% WP (45.00 %) and 0.1 per cent thiophanate methyl 70% WP (41.44 %).

3.3. Efficacy of phytoextracts against *A. porri*

All of the plant extracts evaluated (each @ 10%) *in vitro* were found significantly effective in inhibiting mycelial growth of *A. porri* over untreated control. The data presented in Table 2 revealed that the maximum inhibition (72.22 %) of *A. porri* was recorded with cinnamon extract and was significantly superior over rest of the treatments. It was followed by the extracts of soapnut, jatropha, clove, castor, NSKE, nilgiri, and cassia, with 64.77, 64.44, 46.88, 42.44, 32.44, 30.33, 28.66 and 25.22 per cent inhibition respectively. Whereas the extracts of garlic bulb and sarpaganda were found less effective with the mycelial growth inhibition of 20.33 and 10.88 per cent, respectively.

3.4. Efficacy of bio-agents against *A. porri*

The results presented in Table 3 revealed that *Trichoderma harzianum* (Th) recorded 100 per cent inhibition of *A. porri*, in both cases i.e, centre as well as periphery. *A. niger* (An) also showed complete inhibition of the test pathogen when placed at periphery but when it was placed at the centre, it showed 63.00 per cent inhibition of the test pathogen. *T. viride* showed 48.88 (Tv at centre) and 64.22 (Tv at periphery) per cent inhibition of the test pathogen. *P. fluorescens* was also equally effective as that of *T. viride* and recorded 48.33 per cent inhibition with Bengal method and 41.66 per cent when streaked on two sides. Both the strains of yeast (*S. cerevisiae*) were found least effective against test pathogen.

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4. DISCUSSION

4.1. Symptomatology

4.1.1. Natural / field conditions

On infected leaves small, whitish, sunken, oval shaped lesions were found which later became elliptical or oblong. The lesions were brown to purple at the centre surrounded by a light brown area. Concentric light and dark rings were observed on the infected leaves, when the affected lesion enlarges. In severe cases, blotches were enlarged up to 3 - 4 inches long and covered with conidia. Similar symptoms were also reported by Aveling (1998) and Undhad (2009). Onion blight (purple blotch) earlier reported by Singh (2012), Vijayalakshmi *et al*. (2012) and Wangikar (2012), confirm the present results, compared to symptoms.

4.1.2. Artificial / inoculated conditions

On artificially inoculated onion plant, blight symptoms were visible within 4 - 5 days of inoculation as small, water soaked, sunken and more or less oval shaped spots on leaves. As the disease advanced, the lesions enlarged, became elliptical to oblong, zonated and turned brown to purple surrounded by yellow halo extending upwards and downwards. On the infected area, light to dark concentric rings were developed. The symptoms observed in present findings are in agreement to the earlier reports of Ponnappa (1974) and Koike and Henderson (1998) who recorded similar types of symptoms due to *A. cepulae* on onion and *A. porri* on leek.

4.2. *In vitro* efficacy of the fungicides

All the fungicides tested *in vitro* against the pathogen were found effective in inhibiting the mycelial growth. Propiconazole 25% EC (0.1%) and combi product of iprodione 25% + Carbendazim 25% WP (0.1 and 0.05%) completely inhibited the mycelial growth of *A. porri*. These findings are in agreement with those earlier reported by Sastrahidayat (1994), Singh and Chauhan (1995), Undhad (2009), Chethana *et al*. (2011), Singh (2012) and Vijayalakshmi *et al*. (2012) who reported the fungisides *viz.,* propiconazole, iprodione + carbendazim, difenconazole, tebuconazole, carbendazim + mancozeb, copper oxychloride and mancozeb as most effective fungicides against *A. porri*.

4.3. Efficacy of phytoextracts against *A. porri*

Among the different plant extract testet against *A. porri* maximum per cent inhibition of mycelial growth was achieved with 10 per cent Cinnamon extract (72.22%), followed by Soapnut (64.77%) and Jatropha (64.44%). Results confirm with the findings of Tiwari and Srivastava (2004), Shilpakumari *et al*. (2011) and Wangikar (2012), recorded highest mean mycelial inhibition of *A. porri* with neem (*A. indica*). Similarly, Mishra and Gupta (2012) reported that *Allium sativum* extract @ 10 per cent resulted in maximum inhibition of growth of *A. porri*.

4.4. Efficacy of bio-agents against *A. porri*

Among the various bio-agents tested, complete inhibition of *A. porri* was achieved by *Trichoderma hargianum* and *Aspergillus niger,* followed by *T. viride* and *pseudomonas flourescens.* The present findings are in close conformity with the results of Kharbhari *et al.* (2008) who recorded maximum inhibition of *A. porri* by *T. harzianum*. Similarly, Mishra and Gupta (2012) also reported *T. viride* as most effective in inhibiting with *A. porri*. Undhad (2009) recorded 100 per cent growth inhibition of *A. porri* with *T. viride*-I strain followed by *T. viride*-II (91.75 %). He also reported *T. harzianum*-I and II as antagonistic to *A. porri*.

5. CONCLUSION

1. From the results it is concluded that Propiconazole 25 EC (0.1 %) and combi product of Iprodione 25 WP + Carbendazim 25 WP (0.1 and 0.05 %) are found to be the most effective fungicides *in vitro* against *A. porri* followed by Difenconazole 25 EC (0.1 %), Tebuconazole 5 EC (0.1 %) and combination of Carbendazim 12 WP + mancozeb 63 WP (0.2 %). Mancozeb 75 WP (0.25 %) and Copper oxychloride 50 WP (0.2 %) also resulted in 79.77 and 76.33 per cent inhibition of *A. porri.*
2. Among different plant extracts cinnamon extract (72.22 per cent inhibition over control) is emerged as most effective phytoextracts tested against *A. porri* followed by extracts of soapnut, Jatropha.

Trichoderma harzianum and *Aspergillus niger are found as potential antagonists of A. porri under in vitro* conditions.

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SUMMARY OF RESEARCH

1. Onion, a potential vegetable crop for commercial cultivation, is subjected to be attacked by many pathogens leading to cause serious diseases. Blight (purple blotch) incited by *Alternaria porri* was observed on onion in some pockets of Konkan region of Maharashtra in the range of 14.72 to 26.54 per cent during 2011 to 2013.
2. Fungicides like propiconazole and combi product of iprodione + carbendazim (0.1 and 0.05%), difenconazole, tebuconazole, combination of carbendazim + mancozeb (0.2%), Mancozeb and copper oxychloride can be used for the management of pathogen.
3. Maximum per cent inhibition of pathogen was achieved with plant extracts such as cinnamon, soapnut and jatropha.
4. Bio-agents like *Trichoderma harzianum*, *Aspergillus niger* were found as the potential antagonists of *A. porri*.

DISCLOSURE STATEMENT

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Table 1

In vitro efficacy of the fungicides against *A. porri*

Tr. No.	Fungicides	Conc. (%)	Mean colony dia. (mm)	Inhibition (%)
T_1	Copper oxychloride 50% WP	0.25	21.30	76.33
T_2	Mancozeb 75% WP	0.25	18.20	79.77
T_3	Thiophenate methyl 70% WP	0.10	52.70	41.44
T_4	Propiconazole 25% EC	0.10	00.00	100.00
T_5	Carbendazim 12% + Mancozeb 63% WP	0.20	13.80	84.66
T_6	Tebuconazole 25.9% EC	0.10	12.80	85.77
T_7	Carbendazim 50% WP	0.10	49.50	45.00
T_8	Difenconazole 25% EC	0.10	10.80	88.00
T_9	Iprodione 25% + Carbendazim 25% WP	0.10	00.00	100.00
T_{10}	Iprodione 25% + Carbendazim 25% WP	0.05	00.00	100.00
T_{11}	Control (untreated)	-	90.00	-
	S.Em. ±	-	-	**0.04**
	C.D. (P = 0.01)	-	-	**0.17**

Table 2

Efficacy of plant extracts (10 %) against *Alterneria porri*

Tr. No.	Treatments	Mean colony diameter (mm)	Per cent inhibition over control
T_1	Cinnamon	22.50	72.22
T_2	Jatropha	32.00	64.44
T_3	Castor	51.80	42.44
T_4	Soapnut	31.70	64.77
T_5	Nilgiri	62.70	30.33
T_6	Neem	64.20	28.66
T_7	Neem (NSKE)	60.80	32.44
T_8	Sarpgandha	80.20	10.88
T_9	Garlic	71.70	20.33
T_{10}	Clove	47.80	46.88
T_{11}	Cassia	67.30	25.22
T_{12}	Control	90.00	-
	S.Em. ±		**0.13**
	C.D. (P = 0.01)		**0.51**

*Mean of three replications

Figers in Parentesis are arc sine values

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Table 3

Efficacy of bio-agents against growth of *Alternaria porri*

Tr. No.	Placement details	*Mean colony diameter (mm)	Per cent inhibition over control
T_1	*Ap* *Th* *Ap* *Ap*	00.00	100.00
T_2	*Th* *Ap* *Th* *Th*	00.00	100.00
T_3	*Ap* *An* *Ap* *Ap*	33.30	63.00
T_4	*An* *Ap* *An* *An*	00.00	100.00
T_5	*Ap* *Tv* *Ap* *Ap*	46.00	48.88
T_6	*Tv* *Ap* *Tv* *Tv*	32.20	64.22
T_7	*Pf* *Ap* *Pf*	52.50	41.66
T_8	*Pf* *Ap* *Pf* *Pf*	46.50	48.33
T_9	*Sc-3315* *Ap* *Sc-3315*	80.00	11.11
T_{10}	*Sc-3315* *Ap* *Sc-3315* *Sc-3315*	63.20	29.77
T_{11}	*Sc-3095* *Ap* *Sc-3095*	76.80	14.66
T_{12}	*Sc3095* *Ap* *Sc-3095* *Sc-3095*	60.00	33.33
T_{13}	Control	90.00	-
	S.Em. ±		**0.16**
	C.D. (P = 0.01)		**0.62**

*Mean of three replications

Figers in Parentesis are arc sine values

Where,

Ap = Alternaria porri *Th = Trichoderma harzianum*

Tv = Trichoderma viride *An = Aspergillus niger*

Pf = Pseudomonas fluorescens

Sc= Saccharomyces cerevisiae (NCIM- 3095, NCIM- 3315)

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